

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

<p>Applicant's or agent's file reference see form PCT/ISA/220</p>		<p>Date of mailing (day/month/year) see form PCT/ISA/210 (second sheet)</p>	
<p>International application No. PCT/EP2004/013122</p>	<p>International filing date (day/month/year) 17.11.2004</p>	<p>Priority date (day/month/year) 18.11.2003</p>	
<p>International Patent Classification (IPC) or both national classification and IPC C12N15/82, C12N9/22</p>			
<p>Applicant BAYER BIOSCIENCE N.V.</p>			

1. This opinion contains indications relating to the following items:

- Box No. I Basis of the opinion
- Box No. II Priority
- Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- Box No. IV Lack of unity of invention
- Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- Box No. VI Certain documents cited
- Box No. VII Certain defects in the international application
- Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

<p>Name and mailing address of the ISA:</p> <p> European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016</p>	<p>Authorized Officer</p> <p>Bucka, A Telephone No. +31 70 340-2279</p>
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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - a sequence listing
 - table(s) related to the sequence listing
 - b. format of material:
 - in written format
 - in computer readable form
 - c. time of filing/furnishing:
 - contained in the international application as filed.
 - filed together with the international application in computer readable form.
 - furnished subsequently to this Authority for the purposes of search.
3. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- the entire international application,
 claims Nos. 24-26

because:

- the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
 the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 no international search report has been established for the whole application or for said claims Nos. 24-26
 the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form has not been furnished

does not comply with the standard

the computer readable form has not been furnished

does not comply with the standard

the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

See separate sheet for further details

Box No. IV Lack of unity of invention

1. In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:
 - paid additional fees.
 - paid additional fees under protest.
 - not paid additional fees.
2. This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
 - complied with
 - not complied with for the following reasons:

see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
 - all parts.
 - the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	27-31
	No:	Claims	1-23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-23,27-31

Industrial applicability (IA)	Yes:	Claims	1-23,27-31
	No:	Claims	

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

- D1: TZFIRA TZVI ET AL: "Site-specific integration of Agrobacterium tumefaciens T-DNA via double-stranded intermediates." PLANT PHYSIOLOGY, vol. 133, no. 3, November 2003, pages 1011-1023, XP002319962
- D2: PUCHTA H ET AL: "Two different but related mechanisms are used in plants for the repair of genomic double-strand breaks by homologous recombination" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, May 1996, pages 5055-5060, XP002236852
- D3: PASZKOWSKI J ET AL: "GENE TARGETING IN PLANTS" EMBO JOURNAL, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 7, no. 13, 1988, pages 4021-4026, XP009001858
- D4: WO 03/054189 A (SUNGENE GMBH & CO. KGAA; BIESGEN, CHRISTIAN) 3 July 2003
- D5: WO 96/14408 A (INSTITUT PASTEUR; UNIVERSITE PIERRE ET MARIE CURIE) 17 May 1996
- D6: US-A-5 689 052 (BROWN ET AL) 18 November 1997
- D7: MURRAY E E ET AL: "CODON USAGE IN PLANT GENES" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 17, no. 2, 25 January 1989, pages 477-498, XP000008653

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Present claims 24 to 26 relate to a nucleic acid defined *inter alia* by the sequence of the encoded protein and by reference to the parameter of the GC content of about 50% to about 60%.

In addition, said claims use provisos, i. e. negative features, to define their subject matter. In the case of claim 25, 100 provisos are recited, which make it unduly burdensome to determine the scope of protection sought. The large amount of provisions and conditions in said claims results in a lack of conciseness within the meaning of Article 6 PCT.

The use of the above-mentioned parameter and the use of predominantly negative

features in the present context, which make it difficult to determine the boundaries of the claimed invention, is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters and the multitude of provisos the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful search impossible. As a result, no search report has been drawn up for these claims (Article 17(2)(ii) PCT). Consequently, no written opinion of the International Searching Authority has been established for the subject matter of claims 24 to 26 (Rule 43bis.1(b) in conjunction with Rule 66.1(e) PCT).

Re Item IV

Lack of unity of invention

The following separate inventions/groups of inventions have been identified:

I. claims 1-23

a method for introducing a foreign DNA of interest into a preselected site of a genome of a plant cell comprising the steps of
(a) inducing a double stranded DNA break at the preselected site in the genome of the cell;
(b) introducing the foreign DNA of interest into the plant cell;
characterized in that the foreign DNA is delivered by direct DNA transfer

II. claims 24-31

an isolated DNA fragment comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID No 1 (I-SceI), wherein the nucleotide sequence has a GC content of about 50% to about 60%, and which meets the provisos recited in claim 24

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the reasons outlined below.

Claim 1 relates to a method to introduce a foreign DNA of interest into a preselected site.

Document D1, which is considered to be highly relevant prior art, describes a method for the site-specific introduction of DNA into plants by the formation of double strand

breaks (cf. D1, pages 1012 to 1013).

Document D2, which also describes the site-specific integration of DNA into plants via double strand breaks, refers to an open reading frame encoding the I-SceI enzyme, which is suitable for use in plants (cf. D2, page 5055, right column).

In view of document D1, a first problem to be solved by the present application is considered to be the provision of an alternative method to introduce DNA into plant cells. In view of the disclosure of D1 and D2, a further problem is considered to be the provision of an additional open reading frame encoding the restriction enzyme I-SceI.

The solutions provided by the present application consist of a method to introduce DNA into plants, which is mediated by double strand breaks and which uses direct DNA transfer, and of a further open reading frame encoding the enzyme I-SceI suitable for use in plants, respectively.

) These two problems and their respective solutions are not linked by a common general inventive concept as required by Rule 13 PCT. The transformation method does not require the use of the open reading frame according to claim 24.

Consequently, the application does not satisfy the requirements of Rule 13 PCT.

The application does not contain a single general inventive concept as required to be present by Article 3(4)(iii) and Rule 13.1 PCT. When considering the whole set of claims in the light of the description, no further technical features could be identified which could serve as same or corresponding technical features in the sense of Rule 13.2 PCT to restore unity of invention.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Re Invention I

- 1 The subject matter of present **claims 1 to 23** does not meet the requirements of Article 33(2) PCT. D4 describes a method to target DNA in a site-specific manner to the chloroplast genome of higher plants. In said method, the DNA to be incorporated is introduced by direct DNA transfer, e. g. by particle bombardment (cf. D4, the whole

document).

- 2 Even if novelty could be established, e. g. by reference to the transformation of nuclear DNA, the requirements of Article 33(3) PCT would not be met. Document D2, which is considered to represent the closest prior art, discloses a method for the targeted introduction of DNA into the nuclear DNA of plants cells, in which double strand breaks are induced by the action of the restriction enzyme I-SceI, and in which the DNA to be incorporated is introduced by *Agrobacteria*, from which the subject matter of the present application differs in that it requires the introduction of the DNA into the cell by direct DNA transfer. The problem to be solved by the present application is considered to reside in the provision of an alternative method to target DNA into the plant cells. The proposed solution consists of a method, in which double strand breaks are introduced and in which the DNA to be targeted into the cell is introduced by direct DNA transfer. Document D2 discusses the use of restriction enzymes to induce double strand breaks that facilitate the introduction of DNA into plants. Although the method described in D2 relies on the use of *Agrobacteria* for the transformation, the document explicitly refers to other documents (e. g. D3) discussing the use of direct DNA transfer methods in attempts to target DNA to specific sites (cf. D2, page 5058, right column). In addition, this document raises the hope that the efficiency could be improved by the use of direct DNA transfer methods, as has been described in similar procedures for gene targeting without the induction of double strand breaks (cf. D2, page 5058, right column). For this reason, document D2 provides an incentive for the skilled person to use these direct DNA transfer methods in the protocols described in said document. The person skilled in the art would have followed this proposal with a reasonable expectation to achieve an improvement. Document D1, on the other hand, which describes a highly similar method to introduce DNA into plants by the formation of double strand breaks, emphasizes the double stranded nature of the DNA that is integrated (cf. D1, page 1020). Realizing the importance of double stranded DNA, the skilled person would have considered the use of alternative, routine transformation methods, which use double stranded DNA (e. g. particle bombardment, PEG transformation), in order to introduce the

foreign DNA that is to be targeted to a specific site directly into the plants. As a consequence, an inventive step within the meaning of Article 33(3) PCT cannot be acknowledged for the subject matter of **claims 1 to 23**.

Re Invention II

- 3 Present claim 27 is directed to a nucleic acid with an optimized coding sequence that encodes the I-SceI restriction enzyme.

Document D4, which is considered to represent the closest prior art, refers to a nucleic acid with a sequence encoding the I-SceI restriction enzyme that has been adapted for expression in plants (cf. D4, page 18), from which the subject matter of claim 27 of the present application differs in that it describes a different sequence. The problem to be solved by the present application is considered to reside in the provision of an alternative optimized coding sequence coding for the I-SceI restriction enzyme.

The proposed solution consists of a nucleic acid with a sequence meeting the conditions specified in claim 27.

The need to adapt the original coding sequence of the I-SceI mitochondrial gene to the respective host has been realized in the prior art (cf. D4, page 18; D5, page 13). The adaptation of the coding sequence for expression in monocotyledons has been widely practised in the state of the art (cf. D6, columns 4 to 7), since the differences in codon usage bias among different organisms have been common general knowledge (reviewed *inter alia* in D7). D6 describes such an approach exemplary for the design of a gene encoding an insecticidal protein that is to be expressed in corn. D6 also lists preferred codons and several regulatory motifs that should be avoided in the design of synthetic genes.

It would have been obvious to the person skilled in the art, namely when the same result is to be achieved, to apply the approach used in D6 with corresponding effect to a sequence encoding the I-SceI restriction enzyme according to document D4, thereby arriving at a nucleic acid according to claim 27.

The proposed solution does not show any unexpected or surprising technical effect. Consequently, an inventive step within the meaning of Article 33(3) PCT cannot be acknowledged for the subject matter of **claims 27 to 31**.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/EP2004/013122

- 4 The attention of the applicant is drawn to the fact that the application may not be amended in such a way that it contains subject-matter which extends beyond the content of the application as filed (Article 34(2)(b) PCT).